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The Influence of Calcium on the Refolding of Alpha-Lactalbumin to the Molten Globule State as Evaluated by Time-Resolved FTIR Microscopy

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ABSTRACT: Ordered secondary structures, such as solvated helices, can form on very fast timescales (< 500 microseconds) as shown by our rapid mixing continuous flow IR microscopy investigation of cytochrome-*c* refolding. However, the amide I band profile observed in IR spectroscopy can provide information on a variety of ordered structures (different types of helices and beta-sheets), turn structure, unordered structure and aggregated peptides. Therefore, we want to extend our study of MG formation to alpha/beta proteins. A good candidate protein for such a study is alpha-lactalbumin. The MG state of alpha-lactalbumin forms under acidic conditions in the absence (apo-form) or presence (holo-form) of calcium and contains alpha-helical and antiparallel beta-sheet structure along with turn structure [Troullier *et al*., (2000) *Nat*. *Str*. *Biol*., 7(1): 78-86]. We will acquire equilibrium IR spectra of both apo-form and holo-form ($CaCl_2$ present) MG alpha-lactalbumin under acidic conditions (pD = 2). Prior to performing a refolding experiment, the minimum concentration of urea required to unfold the protein under acidic conditions using CD and IR will be established. For the equilibrium IR studies, labeled urea (^{13}C, ^{15}N) repeatedly lyophilized in D_2O will have to be used to ensure that the amide I band of urea appears below 1600 cm^{-1} away from the amide I absorption of the protein. Once the refolding protocol has been established, unfolded alpha-lactalbumin (pD = 2, in urea) will be refolded to the MG state by rapid dilution using our rapid mixing cell(s) and the influence of calcium on this process will be examined.